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CHANGES OF THE BODY FUNCTIONS DURING LONG-TERM HYPOKINESIA

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16. Abstract (English summary)--Prolonged hypokinesia (100--170 days) studied in 2000 rats kept in cages limiting their mobility provoked considerable changes in the gaseous and energetic metabolism: an elevation of the total gaseous metabolism and of the rate of O_2 requirement by the muscles (in the late periods of hypokinesia). Also, the change in the intensity of tissue respiration of the liver and myocardium. There proved to be a reduction in the level of phosphorylation and separation of oxidative phosphorylation in the myocardium, liver, and partially in the skeletal muscle. Prolonged hypokinesia led to considerable changes in tissue metabolism: a disturbance of development of the animals, a marked delay and an increase in the weight of the organism and the muscular system, and disturbances of the mineral and protein metabolism. Prolonged hypokinesia leads to exhaustion of the hypothalamus-hypophysis-adrenal cortex system.			
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Up until now there has not been a clear understanding of how hypokinesia affects the organism and what kind of changes are most probable during long-term limitation of mobility. It is obvious that this must primarily be the sphere of energetic metabolism because motion has a[^] stimulating effect on all types of metabolism substances and the activity of all systems of the organism in maintaining their tonus. Besides this, it is important to determine the general stability of the organism and resistance of separate tissues.

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Method. Work was completed on 2000 rats weighing 200--250 g. The animals were placed in special locking cages made of plexiglass in which their mobility was sharply limited. The duration of hypokinesia was 60 and 100 days in a series of tests from 120--170. The test and control animals were placed in a single room with uniform food ration (91 kcal per day). Consumption and assimilation of the feed were considered. A chamber method was used for studying total gas exchange. The tissue gaseous homeostasis was determined by a method of tissue gas depots. Analysis of the gas was conducted on a Skalender microgas analyzer. The intensity of tissue breathing and oxidation phosphorylation was determined on slide sections and viscous masses of tissue in the Warburg apparatus. The rate of inclusion of phosphorous (P-32), calcium (Ca-45) and glycine (2C-14) in mineral and protein fraction of bony tissue and teeth which contain specific activity of Ca and P in the bones, the quantity of Ca, Na and Cl generated from the urine, resistance of muscle tissue (according to sorbtion of neutral red by a method of vital coloration) [6]. The content of corticosterone in the blood and adrenal glands, adreno-corticotropic activity of the hypophysis (ACTH of plasma), the

*Numbers in the margin indicate pagination in the foreign text.

reaction of the adrenal cortex to introduction of exogenic ACTH, the response of the adrenal cortex and increase in content of ACTH of the blood during a stress sample (fixing the animal for 15 minutes) were all studied. Functional tests were made for retention of work capability of the organism with determination of the maximum time for remaining afloat and static strength (holding onto a rod). Reserve time when "lifting" rats to 12 km in a pressure chamber was determined as well as endurance to lateral load factors (6 g for 10 minutes in a centrifuge).

Results. The study of total gas exchange showed that the requirement for O_2 in the test rats during the first 70--80 days was somewhat increased over that in the control animals. On days 90--100, this difference became verified. For instance, on day 100, the requirement ^{/4} for O_2 in the test animals was 2.24 ± 0.08 ml/100 g/min, and in the control animals-- 1.83 ± 0.26 ml/100 g/min ($P < 0.05$). Studied (on 200 rats) conducted in different times of the year and on animals with different weight and age confirmed that gas exchange in rats in conditions of limited motor activity is higher than in the control. In certain tests, proven differences were apparent even in the earliest time periods (particularly in the spring) on the 10th to 30th days. However, an increase in gas exchange on days 90--100 detected in all cases was common for all of the tests.

The use of a polarographic method [8] in vivo showed that on days 90--100, in the test animals the rate of consumption of O_2 at separate points on the femur muscle is increased. A constant in the test 0.11 ± 0.18 units per 1 second, in the control 0.069 ± 0.001 ($P < 0.05$). These data attest to the increase in regional gas exchange in the muscles and coincide with the general increase in requirement of O_2 at the end of the test.

The gaseous composition of tissues in the process of long-term hypokinesia is not subject to significant deviation: in the initial period pO_2 31 ± 0.4 mm, pCO_2 47 ± 0.4 mm; on day 100 of hypokinesia, respectively, they are 30 ± 1.5 and 46 ± 2.1 mm. Consequently, in the tissues, no significant changes in intensity of O_2 and CO_2 occur

although the total requirement of O_2 had increased.

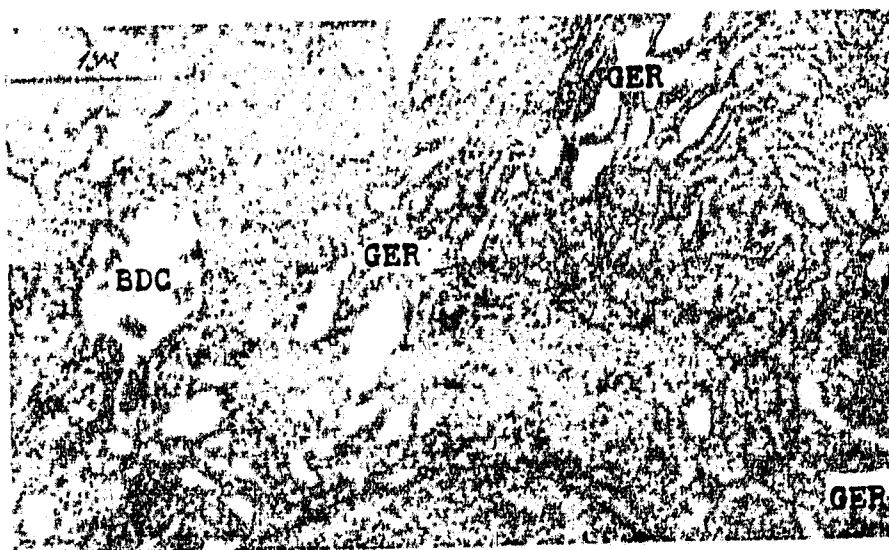
The respiration of certain tissues for the two-month period of hypokinesia changes significantly. During long-term limitation of mobility, always, one detected an increase in intensity of respiration of liver tissue. At first, this was noted when studying the endogenic respiration: the O_2 requirement on day 45 of hypokinesia was increased to 131 ± 10.0 mm³/100 mg of moist tissue in 1 hour at 85 ± 6.0 mm³ in the control group ($P < 0.001$). Later this principle was repeated when studying respiration of tissue in a medium containing different substrata of oxidation: in one test, absorption of O_2 in the liver increased by 50% (day 30 of hypokinesia), and in the other--by 63% (day 45, $P < 0.05$). In the myocardium, this absorption of O_2 on day 60 was decreased from 62 ± 4.8 in the control to 38 ± 6.2 mm/100 mg/hr ($P < 0.05$) in the test (during endogenic breathing). In the tests with respiration with difference substrata of absorption of O_2 by the heart tissue, it decreased from 198.2 ± 7.5 to 164.6 ± 6.6 mm³/100 mg/hr ($P < 0.05$). /5

These changes agree with the results of electron microscope study for the same time periods. In the myocardium during hypokinesia a nonuniform swelling of the mitochondria occurs; the cristae of the mitochondria are less numerous and not as strictly oriented as in the control (Figure 1). Swelling of the endothelium of capillaries occurs and a breakdown in the structure of cellular membranes. Swelling of the mitochondria in this case can be an indication of a certain deficiency of O_2 in the tissues. In the liver (Figure 2) the following are apparent: a strong development of an irregular endoplasmatic reticulum, its close connection with the mitochondria, a change in localization of the irregular reticulum in the cytoplasm--a close approach to the cellular membrane. Such hyperplasia of an irregular reticulum with characteristic expansion of the profile indicates intensification of functional activity of cells--intensification of synthesis of proteins for "external needs." Moreover, accumulation of lipid drops occurs which, however, do not overfill the liver cells but are involved in the process of metabolism of substances. Considering closer contact of lipid inclusions with the mitochondria and granular reticulum and



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Figure 1. Nonuniform swelling of the mitochondria (M) of the myocardium of rats (day 30 of hypokinesia).



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Figure 2. Expansion of the small channels of the granular endoplasmatic reticulum in hepatocytes of rats (day 30 of hypokinesia).

BDC--bile duct capillary; GER--granular endoplasmatic reticulum; N--nucleus, M--mitochondria.

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keeping in mind the presence of bonds between the exchange of lipids and carbohydrates, one can assume that utilization of lipid inclusions is accompanied by synthesis of macroerg bonds which contain phosphorous compounds or acetylcoenzyme A. These processes must be accompanied by intensification of absorption of O_2 so that a definite intensity of liver tissue respiration is established.

It is well known that the character of the relationship of free and phosphorylated oxidation can, to a certain degree, be considered a certain amytal sensitive and amytal resistant respiration, that is, according to the degree of decrease of the requirement of O_2 after injection of amytal into the animal [5]. On day 100 of hypokinesia in animals who cannot move, the amytal resistant respiration amounted to $50 \pm 1.4\%$, and for the control— $47 \pm 2.4\%$, that is, at this time this /6 method did not show a change in the relationship of free and phosphorylated oxidation. However, further study according to the Warburg method indicated that limitation of motor activity caused significant changes in tissue oxidation processes. During the second month of hypokinesia, periods of significant decrease in the level of phosphorylation in the myocardium and skeletal muscle was detected; this can result in a release of oxidation phosphorylation. For instance, in the heart, assimilation of inorganic phosphorous on day 45 of the effect was decreased from 15.4 ± 1.23 (in the control) to 9.5 ± 1.71 $\mu A/100$ m^3/hr ($P < 0.05$) with respiration on succinate and from 24.3 ± 1.95 to 14.3 ± 3.66 μA ($P < 0.05$) with respiration on α -ketoglutarate. On day 60 of hypokinesia, the level of phosphorylation in tissue of the heart also was decreased: 23.1 ± 2.93 in the control and 13.7 ± 2.3 μA in the test ($P < 0.05$). As a result, the P/O coefficient on day 45 was decreased from 1.32 ± 0.11 (control) to 0.77 ± 0.01 in the test ($P < 0.02$).

In the skeletal muscle, the level of phosphorylation also was decreased from 19.9 ± 1.31 to 13.7 ± 1.48 μA ($P < 0.05$) with respiration on succinate and from 19.1 ± 1.30 to 1.5 ± 2.09 ($P < 0.05$) during respiration on α -ketoglutarate (day 45). Later on, on day 100, these changes disappeared: the level of absorption of O and inorganic P in the myocardium and skeletal muscle in the test rats did not differ from the control.

The decrease in level of phosphorylation described above, observed in the heart and muscle tissue during the second month of hypokinesia indicates a slow-down in the process of formation of macro-erg phosphorous compound in these tissues and involves, obviously, a decrease in the functional level of these systems and their "de-training."

Changes in the tissue of the liver also were characterized by a certain decrease in the level of phosphorylation and a breakdown in its conjugation with oxidizing processes, but they occurred wave-like and clearly marked after 30 and 60 days from the beginning of hypokinesia. However, on days 45 and 100 the reverse was observed: a significant increase in the rate of requirement for inorganic P in parallel with intensification of breathing which attests to the intensive function of liver cells. The latter, apparently, involves a breakdown in the exchange processes in the organism during hypokinesia, the predominance of catabolic processes over anabolic and an intensification of disintoxication functions of the liver.

In the test animals, a sharp lag occurred in development and increase in weight. The control animals increased their weight on the average from 183 to 315 g, and those whose mobility was limited for 100 days of the test increased their weight on the average only to 215 g. In another series of tests, 40 rats weighing 300-350 g, after 100 days of hypokinesia, had lost 30--50 g of weight.

On the 80th to the 100th day of hypokinesia, for two groups of animals, the quantity of food consumed, water drunk, feces excreted (for determining the assimilation of the food ration) and the urine generated were all measured. The control animals had eaten on the average in 24 hours 41.6 ± 3.01 g of feed, and the test animals had eaten 45.7 ± 0.93 g. The consumption of food (which consisted of 43% dry substance and 57% water) for the control was 83.2%, for the test--90.1%. Assimilation of the feed in the control and the test was the same--94.8%. Consequently, the decrease in weight increase for the rats during hypokinesia cannot be explained by poor assimilation of food.

The quantity of water consumed by the control animals was 11.8 ± 1.98 ml and in the test animals it was somewhat lower-- 8.55 ± 2.28 ml. The quantity of urine generated in the control was 10.7 ± 2.99 ml, in the test-- 18.6 ± 1.54 ml. Generation of urine in the test animals on days 80--100, was significantly higher than in the control ($P < 0.05$). Consequently, one can note a certain dehydration in the organism of the test rats, but this circumstance does not appear to be the sole cause for their lag in weight. Upon opening up the animal cages on day 110, /7 a noticeable difference was visible in thickness of the subcutaneous fatty layer and the quantity of fat in the internal organs: in the test rats, the amount of fat was considerably smaller than in the control. When weighing the animals, the carcass and muscle of the shin were shown to have considerably slower growth of the muscle system. In the 4th month of hypokinesia, the weight of the test animals on the average was 68.5% of the weight of the control animals, the weight of the carcass --74.3%, the anterior tibial muscle and long extensor of the digits, respectively, 67.5 and 67.4%; the m. plantaris, m. soleus, m. gastrocnemius--respectively, 70.2, 77.2 and 63.9%. The decrease in relative rate of growth in the early period of hypokinesia is greater in the flexor than in the extensor muscles. The absolute weight of the heart, lungs, liver, kidneys, spleen and testicles in the control rats is higher than in the test rats, respectively, by 4.26, 3.4, 23.6, 22.4, 30.9 and 0.5%. A comparison of these weights of separate organs in the weight of the body of a rat (weight index) showed the reverse relationship: the index of weight of most organs in the test rats was higher than the control. For instance, an increase in the weight index of the heart, lungs, liver, kidneys, spleen and testicles in the test rats is, respectively, 30, 50, 6.1, 8.1, 0, 39.4%. Particular attention should be given to the fact that the weight of the heart and lungs becomes much larger than in the control.

The lag in development of skeletal muscles must involve the possibility of fulfilling active dynamic and static work. Actually, dynamic work capability (maximum time for the rat holding onto a load of 15% body weight in the test rats is 5 times lower than in the control (test-- 75 ± 12.6 s, control-- 403 ± 18.2 s). Static work

capability decreased much more sharply. For instance, the time for hypokinetic rats to remain on a vertical rod with notches, on the average, amounted to a total of 1.7--2.5 s, and for the control--93±8.4 s.

The hypoxic sample showed that the "reserve conditional time" (the time before stopping breathing) at an "altitude" of 12 km in hypokinetic rats on the average was 256±57 s (in the control 243±22). Stopping of the heart at this same "altitude" in the test rats occurred after 333±47 s, in the control--after 270±32. Here also there are no verified differences. One would think that a sharp decrease in work capability in a state of hypokinesia would not cause such a change in functioning of systems which deliver O₂ and nutritive substances, as it would changes in the state of the muscle tissue itself. Experimentally it was found that long-term limitation of motor activity of rats is accompanied by progressive decrease in resistance of the muscle tissue. The difference in indices between the test and the control amounted on day 30 to 27%, on day 45--102%, on day 60--65%. According to bibliographical data, the change in resistance in tissue of animals found by a method of vital coloration, is determined by the change in stability of intracellular protein complexes [1, 6]. In conditions of hypokinesia it can be the result of a breakdown in protein metabolism of the tissue [7]. With a 30-day limitation of motor activity of rats, one does not note marked dehydration of tissue in spite of polyuria and increased generation of Na with the urine. Nevertheless, the internal mechanism of water exchange of tissues during hypokinesia, obviously, breaks down; the change in the value of maximal hydration capability is evidence of this [4]. An indirect index of breakdown of the water balance of tissue can also be the increase in sorption capability of the tissue described above (decrease in resistance), inasmuch as it usually is accompanied by an increase in viscosity (thickening) of the cytoplasm [6].

As was already noted, long-term hypokinesia in humans is accompanied by increased removal of Ca from the organism. A study of the state of /8 the support-motor apparatus in conditions of limitation of mobility is

of interest still and therefore recently attention has been given to the presence of the intrinsic piezo-electric effect in bony tissue caused by the constant effect of load on the bones with active muscle contractions [2]. The latter can be used as a constant source of intrinsic electrophysiological stimulation of the trophics of bony tissue during normal metabolism in it. Our study showed that in the test animals by day 100, a proven decrease had occurred in the inclusion of Ca-45 in the femoral bones by 14--20%, and specific activity of Ca-45 in the femoral bones has decreased by 15%. The rate of inclusion of P-32 is decreased in the mineral fraction of the femoral bone by 24%, and specific activity of P-32 is decreased by 25.7%. Inclusion of glycine-2C-14 which comprises the base of protein matrices of bony tissue, in proteins of calcified tissues is decreased by 22.5%. In the growth zone of the roots of incisors in rodents, the process of constructing protein structures and their calcification is constantly being completed. The inclusion of glycine-2C-14 in the proteins of the incisors is decreased in the test rats by 15% and inclusion of P-32 in the incisors by 31%. The specific activity of P-32 in the teeth is weakened by 30.9%. A breakdown in exchange of Ca whose metabolism is more stable follows these changes. In animals who are not allowed to move, on day 90--100 of the test, output of Ca had increased by 44%. An increase in the output of Cl and Na with the urine was established. As a result of these changes in mineral exchange, there can be a disturbance in strength of the support-motor apparatus, the structure of the teeth, and also normal development and growth of the organism.

The first 30--35 days of the test were accompanied by sharp intensification of synthesis and secretion of corticosterone (in the blood-- 27.58 ± 0.71 $\mu\text{g}\%$ with 10.8 ± 0.61 in the control, and in adrenal glands-- 25.7 ± 1.35 with 8.3 ± 0.15 in the control). Then right up to day 60, there followed a period of normalization of functions of the adrenal cortex and content of ACTH in the plasma, having shifted the phase of impoverishment of the system. On day 170, the content of corticosterone in the blood and adrenal glands and ACTH in the plasma had decreased by almost two times. The first phase of intensification of the function of the hypophysis and adrenal cortex system has a very unusual

character: we noted two waves of increase in the content of hormones in the blood and adrenal glands on days 1--3, 12, 15 and 18 of hypokinesia. The tests with trained animals made it possible to conclude that the first wave is the result of emotional stress and the effect of limited mobility is apparent at the end of the second week.

The adrenal cortex up to 60 days retains its capability to adequately respond to the injection of exogenic ACTH. At the same time, in response to a stress test, the increase in level of corticosterone in the adrenal glands and ACTH in the plasma is noticeable to a lesser degree than in the control animals (respectively, 39.9 ± 1.17 $\mu\text{g}\%$ with 46.9 ± 0.61 in the control and 4.07 ± 0.24 units/100 ml at 4.29 ± 0.8 in the control). This attests to the fact that although the base level of corticosterone and ACTH in the blood and adrenal glands on day 60 of hypokinesia varies in normal limits and the functional possibilities of the adrenal cortices are retained, however, in higher sections of the system of hypothalamus-hypophysis-cortex of the adrenal glands, changes even occur which are characterized by a decrease in their reaction to extreme effects. In later time periods, the response of the hypophysis and adrenal cortex system on the introduction of ACTH and the stress effect is decreased and on day 130 it is practically absent. The studies of these parameters, after stopping the effect of hypokinesia lasting 60 and 130 days, confirmed the principles described above of a breakdown in functioning of the system studied. After 60-day hypokinesia, the function of the hypophysis-adrenal system is fully recovered after 20 days; after the 130-day effect, even at the end of the second month, normalization of activity of this /9 system is not detected. Here, only the basal level of the corticosterone in the blood and adrenal glands was recovered; the content of ACTH in the plasma remained low (0.76 ± 0.6 units/100 ml); the reactivity was decreased by 40%. The latter also confirms the conclusion as to the large range of the upper sections of the endocrine regulators.

It follows from the data obtained that the model we used of hypokinesia is at least a two-component stressor (emotional and metabolic) which depletes the hypothalamus-hypophyseal-adrenal system and the adaptive potential of the organism.

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